A COMPARISON OF COMBINATION OF RECOMBINANT GONADOTROPHIN AND CLOMIPHENE CITRATE PROTOCOL WITH RECOMBINANT GONADOTROPHIN ALONE PROTOCOL AS OVARIAN HYPERSTIMULATION AGENTS IN INTRAUTERINE INSEMINATION CYCLES IN HOSPITAL SULTANAH NUR ZAHIRAH: A PILOT STUDY

By

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LIST OF ABBREVIATIONS

ART	-	Assisted reproductive technique
BMI	-	Body Mass Index
CC	-	Clomiphene Citrate
COH	-	Controlled Ovarian Hyperstimulation
CRF	-	Clinical Research Form
DNA	-	Deoxyribonucleic Acid
ET	-	Endometrial thickness
FSH	-	Follicle Stimulating Hormone
GnRH	-	Gonadotrophin Releasing Hormone
hCG	-	Human Chorionic Gonadotrophin
hMG	-	Human Menopausal gonadotrophin
HSNZ	-	Hospital Sultanah Nur Zahirah
HUSM	-	Hospital Universiti Sains Malaysia
i.e.	-	*id est*, that is
IM	-	Intramuscular
IU	-	International Unit
IUGS	-	Intrauterine gestational sac
IUI	-	Intrauterine Insemination
IVF	-	In vitro fertilization

LH	-	Luteinizing Hormone
mg	-	milligram
MHz	-	Megahertz
Miu/ml	-	Mili international unit/ milimiter
Mm	-	milimeter
OHSS	-	Ovarian Hyperstimulation Syndrome
PCOS	-	Polycystic Ovarian Syndrome
PR	-	Pregnancy Rate
RCOG	-	Royal College of Obstetricians and Gynaecologists
RCT	-	Randomised control trial
rFSH	-	Recombinant Follicle Stimulating Hormone
SC	-	Subcutaneous
SFA	-	Seminal fluid Analysis
TVS	-	Transvaginal scan
uFSH	-	Urinary follicle Stimulating Hormone
uFSH-HP	-	Highly purified urinary Follicle Stimulating Hormone
UK	-	United Kingdom
UPT	-	Urine Pregnancy Test
vs.	-	Versus
WHO	-	World Health Organization

ABSTRAK

OBJEKTIF: Satu kajian penyelidikan dijalankan di Hospital Sultanah Nur Zahirah untuk menilai tahap keberkesanan di antara dua protokol stimulasi minima menggunakan gabungan ubat clomiphene citrate dan rFSH dan ubat rFSH sahaja di dalam prosedur stimulasi ovari secara terkawal dalam Teknik Permanian Beradas. **KAEDAH KAJIAN**: Satu kajian prospektif rawak terkawal yang dijalankan di Klinik Infertiliti Hospital Sultanah Nur Zahirah bermula dari 1^{hb} Oktober 2013 hingga 30^{hb} September 2014. Kajian ini melibatkan 68 orang pesakit yang berumur 24 hingga 44 tahun yang menjalani kaedah stimulasi hormon dan permanian beradas kali pertama dengan protokol stimulasi minima. Mereka telah dipilih secara rawak, di mana 34 orang pesakit menerima protokol gabungan ubat clomiphene citrate dan rFSH, manakala 34 orang pesakit lagi menerima protokol ubat rFSH sahaja. Perbandingan telah dibuat ke atas keberkesanan kedua-dua protokol ini dari segi pembesaran dan bilangan folikel terhasil dan kadar kehamilan. Selain itu, ketebalan dinding rahim terhadap stimulasi ubat subur yang diberikan dan peratusan komplikasi kehamilan seperti keguguran, kehamilan kembar atau sindrom 'ovarian hyperstimulation' juga dikenalpasti. **KEPUTUSAN**: Kajian ini mendapati tiada perbezaan yang signifikan di antara protokol gandingan CC dan rFSH dan protokol rFSH sahaja dari segi sosio-demografik, antropometrik, tempoh, sebab dan jenis infertiliti dan analisa sperma. Ketebalan dinding rahim pada kumpulan yang menerima protocol CC-rFSH adalah 8.5mm dan kumpulan rFSH sahaja adalah 10.1mm. Perbezaan ini adalah signifikan daripada segi statistic ($p<0.05$). Purata bilangan folikel pada kumpulan CC-rFSH adalah 1.79, manakala purata bagi kumpulan rFSH sahaja adalah 1.44, dimana perbezaan ini tidak signifikan. Kadar kehamilan untuk kumpulan menerima kedua-dua protokol stimulasi minima tidak menunjukkan perbezaan yang signifikan dengan peratusan 11.8% berbanding 8.8%

dengan kadar p=0.690. Tiada kes kehamilan kembar atau insiden sindrom ‘ovarian hyperstimulation’ dalam kajian ini. Dari segi komplikasi kehamilan seperti keguguran, kadar insidennya adalah sangat rendah dan tiada perbezaan signifikan di antara kedua-dua kumpulan kajian. **KESIMPULAN**: Secara keseluruhannya, protokol stimulasi minima dapat mengurangkan komplikasi seperti kandungan kembar dan sindrom ‘ovarian hyperstimulation’. Walaubagamana pun, kedua-dua protocol minimal dalam kajian ini mempunyai tahap keberkesanan yang sama dari segi kadar kehamilan. Kedua-dua protokol ini boleh digunapakai, dan pemilihan protokol adalah unik untuk setiap pasangan dan bergantung kepada faktor infertiliti yang dialami oleh pesakit.

ABSTRACT

OBJECTIVE: A study was performed in Hospital Sultanah Nur Zahirah to evaluate the efficacy of two minimal ovarian hyperstimulation (MOH) protocols between a combination of clomiphene citrate and recombinant gonadotrophin and recombinant gonadotrophin alone in controlled ovarian hyperstimulation for intrauterine insemination (COH-IUI). **METHOD**: A prospective randomized controlled trial was conducted at the Infertility Centre of Hospital Sultanah Nur Zahirah for one year duration from 1st October 2013 until 30th September 2014, aged between 24 to 44 years old women, undergoing their first COH-IUI cycle with MOH protocols. 68 women were randomly assigned to each protocol, with 34 women received CC-rFSH protocol and 34 women with rFSH alone protocol. The primary outcomes were to compare the effectiveness of both protocols in term of pregnancy rate and follicle development in term of number and sizes of dominant follicles. In addition, the endometrial thickness in response to each protocol and the incidence of complications related to COH-IUI such as miscarriage, multiple pregnancy and OHSS were also evaluated. **RESULT**: There were no statistical differences noted between CC-rFSH protocol and rFSH alone protocol in term of sociodemographic, anthropometrics, duration, cause and type of infertility and also sperm counts, motility and morphology, suggestive that the subjects were homogenously distributed. The endometrial thickness of CC-rFSH protocol was 8.5mm, whereas in rFSH alone protocol was 10.1mm. The difference was statistically significant with p value of <0.05. The difference of mean number of follicles was not significant between the two protocol, with 1.79 in CC-rFSH protocol and 1.44 in rFSH alone protocol. The pregnancy rate between the two protocols was not statistically significant with 11.8% versus 8.8% (p value=0.690). There were no cases of higher

order pregnancy and no reported incidences of OHSS. There was only 1 case of first trimester miscarriage, but statistically did not achieve any significant difference between the protocols. **CONCLUSION**: Both minimal ovarian hyperstimulation protocols are equally effective for COH-IUI in term of pregnancy rate with lower incidence of complications. Adoption of both protocols is recommended, and the choice of protocol should be individualised and uniquely chosen to each couple with different causative factor for infertility.

1.0 INTRODUCTION

Infertility is defined as failure to conceive after frequent unprotected sexual intercourse over a period of one year. Presently, numerous modes of assisted reproductive techniques (ART) are widely used to treat infertility. Intrauterine insemination (IUI) is the most commonly used ART to treat infertile couples with unexplained or male subfertility and it is normally used in combination with controlled ovarian hyperstimulation (COH). This combination has resulted in significantly higher cumulative pregnancy rate per couple as compared to unstimulated intracervical insemination, COH alone or IUI alone.

The main goal of COH is to achieve multifollicular growth as it is associated with higher pregnancy rate (PR) compared with monofolllicular growth. However it is associated with complications particularly multiple pregnancies and ovarian hyperstimulation syndrome (OHSS). The incidence of multiple pregnancies after COH-IUI is estimated to be 10-40% per cycle and that 30-50% of all multiple pregnancies is due to COH-IUI (Fauser et al, 2005). Multiple pregnancy is classified as high risk pregnancy due to significant neonatal and maternal morbidity. In OHSS, the incidence is around 2% of assisted conception treatment cycles and the risk is increased in the presence of PCOS and younger age women (Aboulghar and Mansour, 2003).

Over the years, various agents are used to achieve ovarian hyperstimulation in ART, which include the Human Menopausal Gonadotrophin (hMG), purified FSH, recombinant FSH (rFSH) and anti-estrogens, such as clomiphene citrate (CC) and aromatase inhibitors. Various protocols have been used using these agents or combination of these agents. The effectiveness of each protocol was evaluated in order to find the best protocol carrying high success rates with minimal complications, thus the formation of minimal ovarian hyperstimulation (MOH) protocol. Mono-ovulation with lesser complication was observed using the MOH protocol. Therefore, it was suggested that patients undergoing IUI cycles may be benefited more using such protocol.

Various regimes have been suggested for the MOH protocols. However, only small studies comparing the effectiveness of each regime are available. In Hospital Sultanah Nur Zahirah, the combination of CC and recombinant gonadotrophin has been used in the MOH protocol. Its effectiveness is yet to be evaluated. Therefore this study was done to compare the efficacy of current protocol (CC + recombinant gonadotrophin) with a new recommended low-dose gonadotrophin-alone protocol. Should it be shown to be more effective, this new protocol could be used as the standard COH protocol in HSNZ.

2.0 LITERATURE REVIEW

2.1 PHYSIOLOGY OF GONADOTROPHIN HORMONE AND OVULATION

According to the two cell theory, in normal ovarian follicular development, the follicular stimulating hormone (FSH) is necessary for recruitment of the follicles and stimulation of the granulosa cell, which is responsible for selecting the dominant follicles. The luteinizing hormone (LH) stimulates the androgens, which are subsequently aromatized to oestrogens by granulosa cells under the influence of FSH stimulation. This synergistic action of FSH and LH results in rising oestrogen level in the follicular fluid. This oestrogen will in turn influence the rate of follicular growth. Ovulation results when there is an appropriate physiologic gonadotrophins secretion from pituitary gland and also normal regulations of pulsatile release of gonadotrophin releasing hormone (GnRH) from the hypothalamus. The actions of FSH and LH are summarised as in Table 1 (Speroff L et al, 1994).

Table 1: Mechanism of Action of FSH and LH

Follicle Stimulating Hormone	**Luteinizing Hormone**
-Granulosa cell membrane binding **- Activation of adenylcyclase system** **- Synthesis of cytochrome P450 aromatase** **- Induction of LH receptors** **- Enzyme activation for P synthesis**	**- Provide androgen substrate for FSH-induced aromatase** **- Resumption of oocyte meiosis Cumulus oophorus maturation** **- Follicular rupture** **- Corpus luteum formation and support**

Significant alterations of either GnRH or FSH/LH secretion pattern leads to anovulation. It will also lead to excessive circulating LH, which leads to elevated androgens and promotes follicular atresia. This androgenic environment is not conducive for normal folliculogenesis which is commonly seen in conjunction with polycystic ovarian disease (PCO).

The administration of gonadotrophins can override normal ovulatory mechanisms for ovarian follicullogenesis. Ovarian stimulation improves the cycle fecundity rate in part by the number of follicles available for fertilization and correcting subtle, unpredictable ovulatory dysfunction. Combined with IUI, ovulation stimulation is recommended for many causes of infertility with patent tubes (Hughes EG, 1997).

2.2 CONTROLLED OVARIAN HYPERSTIMULATION

Controlling the number of growing follicles is one of the key issues in ovarian stimulation in women undergoing IUI as the number of follicles on the day of human chorionic gonadotrophin (hCG) administration is the main determinant of both pregnancy rate and risk of complications. The introduction of MOH has shown to reduce the risk of OHSS and multiple pregnancy and it costs lesser as the reduce dosage of gonadotrophin is used in a shorter cycle.

2.3 DRUGS USED FOR COH

The first choice of drug for COH-IUI is still remain debatable. Various studies on MOH protocols produced conflicting results when comparing the efficacy of CC with different types of gonadotrophins in IUI cycles. COH with rFSH preparation in low dose protocols has been shown to produce mono-follicular development, reduce the rate of twins without affecting the overall PR.

2.3.1 Clomiphene citrate (CC)

The success of the usage of CC as ovulation induction agent with an improved conception rate was first reported in 1961. It has been extensively used in infertile patients and continued to be the most commonly used ovulation agent since 1967 when it was approved by the United States Food and Drug Administration (FDA). In 2007, Dankert T. et al suggested CC as the first line drug as it carried same efficacy like other agents and was cost effective.

CC is a non-steroidal synthetic oestrogen. Its primary site of action is at the level of hypothalamus and pituitary, in which it occupies the oestrogen receptors, thus blocking the negative feedback action of oestradiol. As a result, there is a rise in serum FSH concentrations and subsequently stimulates the follicular growth and follicular oestradiol production. However, due to its anti-oestrogenic effect, the use of this drug is associated with detrimental effect on the endometrium and suppression of cervical mucus.

2.3.2 Gonadotrophin Hormone (GnRH)

Ovulation induction using exogenous gonadotrophins were successfully used in treating women with infertililty problems and an increment of fecundity rate was achieved by 15-30%. Women with chronic anovulation and fail to ovulate with clomiphene citrate are candidates for treatment with this drug.

Various FSH- containing products, either derived from extraction and purification from urine or recombinant in vitro technology, have been developed in these years. FSH containing gonadotrophin preparations can be divided into four groups; (i) hMG, containing both FSH and LH; (ii) urinary FSH (uFSH); (iii) highly purified urinary FSH (uFSH-HP); and (iv) rFSH.

hMG was first derived in Italy in 1940s, extracted and purified from human post menopausal urine, containing equivalent amounts of urinary human FSH and of human LH (FSH 75IU and LH 75 IU). It also contains large quantities of potentially allergenic urinary proteins. Later, in 1959, the purified urinary FSH was produced by removing the LH component by a purification process of hMG. Stranger J.D et al, 1985 reported that presence of significant amount of LH was thought to lead to poor oocyte quality, reduced fertilization rates, lower embryonic viability and early pregnancy loss. Apart from that, it contains small amounts of urinary protein and it diminishes adverse reaction such as local allergy or hypersensitivity.

Urofollitropin, has been made available since the mid 1980's, and approximately 10 years ago, the preparation is made practically devoid of LH activity (FSH 75 IU and LH <0.7 IU). It was developed to replace hMG in ART services (Jean-Yves et al, 1998).

However, there were concerns about both hMG and uFSH preparation as it contains undefined, urinary protein contaminants, rendering their purity <5%. Bruno L (2004) stated in his study that through the application of immunochromatography with monoclonal antibodies against FSH, an increased purity of >95% was achieved in highly purified uFSH preparations.

In 1990s, in vitro production of large quantities of human FSH and suitable for pharmaceutical use through recombinant DNA technology has been achieved. Recombinant FSH thought to represent the ultimate solution in ovulation induction, which is completely free of LH and extraneous human proteins, which is more than 99% pure FSH. These advantages consist of being independent from urine collection, ensuring a constant FSH supply and guaranteeing a batch to batch consistency.

The human rFSH, in the form of follitrophin α and β are derived from genetically engineered cell line of mammalian i.e. the Chinese hamster ovary cell line. The genes encoding the α- and β- FSH subunits were isolated from a gene bank. The functional genes were then introduced into the genome of the Chinese hamster ovary cell line.

The selected recombinant cells synthesize the subunits of FSH and secrete a glycosylated bioactive dimeric FSH. The amino acid sequences of the recombinant α

and β- subunits are identical to the predicted amino acid sequences obtained from the nucleotide sequence α and β- subunits of DNA genes. Puregon® manufactured by Organon, Netherlands, is follitropin beta, which is an example of rFSH. It contains 5 micrograms, equivalent to 50 IU/ml of this substance. The powder and solvent for solution for injection is in a pre-filled syringe. It is intended for subcutaneous administration.

2.3.3 Aromatase Inhibitor

Aromatase inhibitors (AIs) have been used in the treatment of patients with anovulatory infertility, such as PCOS, and for increasing the number of ovarian follicles recruited in ovulatory women undergoing COH. Aromatase activity is present in many tissues including ovaries, brain, adipose tissue, muscle, liver and breast. Aromatase is a microsomal cytochrome P450 haemoprotein-containing enzyme that catalyses the rate-limiting step in the production of oestrogens; the conversion of androsthenedione and testosterone via three hydroxylation steps to oestrone and oestradiol, respectively. This leads to low level of serum oestrogen and subsequently reduces the negative oestrogenic feedback at the pituitary. It causes increased in FSH secretion that stimulates the development of ovarian follicles. It is also known to increase intrafollicular androgen which in turn is thought to upregulate and sensitize FSH receptors in the ovary. AI, unlike CC, does not decrease the oestrogen receptor density or thin the endometrial lining.

Letrozole is selective AI and its effect is reversible. It gained its original approval from FDA in 1997 for the treatment of breast cancer in menopausal women. It was first used

in infertility treatment in anovulatory women in 2001. The first randomised trial comparing CC with letrazole for women with unexplained infertility (Sammour A et al, 2001) showed thicker endometrium and higher pregnancy rate for the letrazole group compared to CC group. AIs are the ideal choice when a limited number of follicles are required or there is a risk of hyperstimulation syndrome.

Recently, the safety of letrazole was seriously questioned after an abstract was presented in a meeting in 2005, suggested that the use of letrazole for infertility treatment might be associated with a higher risk of congenital cardiac and one malformations in the newborns (Biljan et al, 2005). However, there is no evidence that the exposure of oocyte to letrazole can increase birth defect. It has been shown that AIs and CC are equally effective in inducing and augmenting ovulation as well as resulted in favourable pregnancy outcomes.

2.3.4 Tamoxifen

Tamoxifen is a non steroidal selective oestrogen receptor modulator (SERM). SERM are thought to act primarily by binding with oestrogen receptors at the hypothalamus. This competitive inhibition results in a perceived drop in endogenous oestrogen levels, eventually leading to increased gonadotrophin secretion and subsequent induction of ovulation. Although Tamoxifen is commonly used today as an adjuvant therapy in the treatment of breast cancer, its usage as ovulatory agent was first reported in 1973 by Williamson and Ellis. Unlike clomiphene, Tamoxifen acts as an agonist on the oestrogen receptors of vagina mucosa and endometrium. It has been used to induce ovulation with an equal effectiveness as CC (Boostanfar et al., 2001).

Its limited usage could be due to the potentially serious side effects and the long term risks of treatment. The side effects are hot flushes, vaginal discharge, menstrual irregularities, fluid retention fatigue and depression. The other serious side effects and long term risks that are rare, but are more dangerous include endometrial hyperplasia, endometrial polyps, endometrial cancer, venous thromboembolism, stroke, ovarian cysts and cancer and cataract formation.

2.4 REGIMES OR PROTOCOLS IN MOH

The goal of ovulation induction for World Health Organisation (WHO) Group II anovulatory women is to achieve mono-ovulation. Ovarian stimulation with rFSH, used in MOH protocol has shown to be successful in producing mono-follicular development in more than half of the women undergoing therapy. The MOH protocol minimises the risk of multiple follicular development and the associated risks of OHSS and multiple pregnancy (Homburg and Howles, 1999). Various regimes have been proposed in the literatures.

2.4.1 CC versus rFSH

CC is mainly used in women with anovulatory infertility. The treatment is usually commenced for 5 days from the second day of menstrual bleeding until the sixth day. It is normally started with the lowest dose of 50mg and an increment of 50mg per cycle is required in the anovulatory cycles, until presence of growing follicle or a maximum dose of 250mg per day is achieved. The discrepancy between ovulation and pregnancy

rates could be attributed to the anti-estrogenic effect of the drug on the endometrial lining and cervical mucus, and its probable action on tubal transport and uterine blood flow. A randomized multicentre trial using a parallel design comparing the efficacy of CC with rFSH in primary unexplained or male subfertility undergoing IUI cycles has found no significant difference in live birth rates between the two groups (Dankert et al, 2007).

2.4.2 Different gonadotrophin preparations used in MOH

A comparison of effectiveness of different gonadotrophin preparations in IUI cycles for patients with unexplained infertility was performed on two hundreds and forty one patients has found that rFSH resulted in better outcomes in IUI cycles compared to other preparations such as uFSH and hMG (Demirol A and Gurgan T, 2007).

2.4.3 Different dosages of rFSH

Low-dose approach requires less monitoring because of lower risks of multiple pregnancy and OHSS and is less costly. Therefore this approach improves access to treatment for infertile couples with financial restraint. A study of three low-dose regimes of rFSH in MOH had shown no significant differences among the three protocols in term of cycle parameters (Hughes et al, 1998).

Pregnancy rate with low dose gonadotrophin protocols do not significantly differ from more aggressive stimulation protocols, and these low-dose protocols are associated with significantly fewer side effects (Sengoku et al, 1999).

2.4.4 The duration and timing of FSH administration

Timing of FSH administration is critical to control number of recruited follicles following ovarian stimulation (Cedrin-Durnerin I et al, 2006). The aim for a limited number of large follicles was more successfully achieved in patients whose rFSH given in the mid to late follicular phase than in early phase. If given too early, multifollicular development will occur, thus the supplementation of rFSH during mid to late cycle is recommended.

Moreover, daily injection of rFSH is recommended rather than alternate days in achieving the optimum result (Cedrin-Durnerin I et al, 2006).

2.5 COMPLICATIONS OF OVULATION INDUCTION

Complications of COH include OHSS, multiple pregnancy, pregnancy wastage and increased incidence of heterotopic pregnancies. A major complication associated with the use of ovulation agents is the occurrence of OHSS. All complications are essentially related to degree of ovarian stimulation during ovulation induction. Fortunately with careful clinical, ultrasonographic and biochemical monitoring, the degree of severity and the frequency of complication can be reduced significantly.

OHSS occurs due to excessive ovarian response following the luteinisation initiated with hCG. It is characterised by a cystic enlargement of the ovaries and an acute fluid shift from the intravascular to the third space, which causes ascites, pleural effusion, pericardial effusion and intravascular dehydration. The risk of OHSS can be reduced by choosing milder form of ovarian stimulation for ovulation induction aiming for unifollicular induction using low dose gonadotrophins.

Multiple pregnancy becomes one of the major adverse effects of ovarian stimulation as it is associated with increased risk of miscarriage, preterm delivery, low birth weight, pre-eclampsia, gestational diabetes mellitus and complicated labour. Low-dosage protocols have been recommended to avoid multifollicular ovarian response, hence reducing the incidence of multiple pregnancy.

A few studies had investigated the relationship between multiple pregnancy and number of follicles induced in COH-IUI. Induction of more than one follicle did not improve the PR significantly, but increased the risk of multiple pregnancy (Van Rumste et al, 2006; Nuojua-Huttunen et al, 1999).

2.6 INTRAUTERINE INSEMINATION (IUI)

IUI is a well known cost effective low technology ART for infertility, but as compared to IVF, not many studies were done to compare the effectiveness, efficacy and cost effectiveness of various ovulation agents for induction in IUI. There are wide variations in indications, protocols of ovarian stimulation, semen preparation, timing, number and technique of insemination. IUI is a technique to assist the placement of the spermatozoa

that have been processed from the seminal fluid into the endometrial cavity through a small catheter. IUI alone or in combination with COH has helped many infertile couples in achieving pregnancy.

2.6.1 Indication for IUI

COH-IUI is indicated in women with unexplained infertility, prolonged subfertility, cervical factor infertility, or stage I or II endometriosis and in women with single fallopian tube (Table 2). Contraindications for COH-IUI include ovarian failure, significant presence of male factor, significant tubal adhesions or tubal dysfunction or significant uterine abnormalities.

Table 2: Indications of IUI

Female factors	Male factors	Others
1.Anatomical defects of vagina or cervix **2.Hostile cervical mucous** **3. Sexual dysfunction** **4. Mild to moderate endometriosis** **5. Endocrine causes** **6. Anovulatory cycle**	**1. Anatomical defect of penis** **2. Ejaculatory or erectile dysfunction** **3. Retrograde ejaculation** **4. Immunological factor** **5. Oligospermia**	**1. Unexplained (all available investigations for both couples are normal)**

2.6.2 Monitoring of ovarian response

In term of follicular monitoring, apart from ultrasonic follicular measurement, preferably it should include serial serum oestradiol level as well. Clinical monitoring of cervical mucus production is less precise and insensitive in achieving the above goals.

Ultrasonography has been used to assess follicular growth during normal menstrual cycles and during gonadotrophin injection. Follicular growth was noted to be linear during the ultrasonic examination and they appeared to have strong correlations between follicular growth and oestradiol measurements. However in the literature, the best method available for ovulation prediction, detection and confirmation is yet still being debated. Ovulation detection is fundamental in diagnostic work-up of infertile couples and to decide on optimal artificial insemination timing and ultrasound is still standard monitoring tool for follicular tracking in relation to IUI procedure.

Transvaginal ultrasound is the best method to evaluate the size of follicles and discriminate between single and multiple follicular growths. Prevailing evidence suggest that the follicles of 16-22 mm will likely to ovulate (Seibel MM et al, 1981*).* The PR was related to the number of follicles that were ≥ 15 mm at the time of hCG injection and Dickey et al (1992) reported the pregnancy rate was 17.7% per cycle when there were 3 or more preovulatory follicles. Women with follicular diameter of ≥ 20 mm were 40 % less likely to become pregnant as compared to women with diameter between 15.00 and 19.99 mm (Ghosh et al, 2000).

It is also useful to predict the endometrial receptivity. Two parameters are used for evaluation; endometrial thickness (ET) and endometrial pattern. A presence of triple line multilayered pattern during ultrasonography reflects better endometrial receptivity. ET measurement has been used to predict implantation following induction in both, IVF and non-IVF cycles. A study by Christian De Geyter et al in 2000 revealed that PR in patients with a thin endometrium were equals to those with normal endometrium. It is of interest that the lowest reproducibility was found in patient with thick endometrium (> 11 mm). Based on previous study, the lowest significant cut off value or endometrial thickness was varied from 6-9 mm.

A study on the evaluation of efficacy of three different cut off levels of serum progesterone concentrations in three different occasions of blood samples in mid-luteal phase was done to confirm ovulation. Its role is to determine the best day of the cycle for serum progesterone measurement. The results had consistently showed good reliability of that method for confirming ovulation. One progesterone assessment in the midluteal phase seems highly effective for confirming ovulation (Guermandi et al, 2001). Values most reported as indicative of ovulation are those that exceed 16 nmol/L (equivalent to 5 ng/mL) for a minimum of 5 days, or a single value exceeding 32 nmol/L (corresponding to 10 ng/mL) in the midluteal phase. Peter Platteau et al in 2006 suggested that midluteal progesterone concentrations of less than 10 ng/mL is associated with lower pregnancy rate per cycle than progesterone levels above 10 ng/mL and ideally the sample should be taken during the midluteal phase (6–9 days after HCG administration).

Study by Wan Rosmidah et al (2006), regarding follicular rupture at time of IUI revealed that 74.8 % of follicle had ruptured at the time of insemination following human chorionic gonadotrophin (hCG) administration with the pregnancy rates were 12.6 % but there was no significant difference of pregnancy rate in both group either ruptured or unruptured follicle at the time of IUI. The highest rate of ruptured follicle were observed among those couples with unexplained infertility, number of dominant follicle of more than 4 and those who had IUI done after day 15 with rate of 91.3 %, 82.8 % and 93.7 % respectively. The type of infertility and endometrial thickness did not significantly associated with the rate of follicular rupture.

2.6.3 Sperm Preparation

Sperm preparation in IUI procedure is important. Sperm washing allows selection of sperm with the normal morphology and motility and with the absence of antibodies, white blood cells, and infectious organisms. The sperm preparation yielding the largest population of highly motile cells free of other cellular and chemical components of seminal fluid is preferred. The choice of technique is based on the initial quality of the sample. The most commonly used methods are the standard swim-up and density gradient preparations. Both methods yield similar cycle fecundity rates (Dodson WC et al, 1998).

2.6.4 Intrauterine Insemination

The timing of IUI in relation to ovulation strongly influences the chance of conception. Follicular stimulation with CC or Gonadotrophin followed by administration of hCG, will promote final follicular maturation leading to follicular rupture and release of oocyte and formation of corpus luteum. In spontaneous cycle, the onset of the LH surge appears to be the most reliable indicator of impending ovulation occurring 34-38 hours prior to ovulation as reported by Testart et al in 1982. Due to the biochemical similarity between hCG and LH in ovulation induction, it has been assumed that ovulation occurs 36-38 hours after the hCG administration (Speroff L et al, 1994).

Fischer et al in 1993 had found that follicular rupture occurs over a broad range of time (36-48 hours) after hCG administration, whereas Testart J et al in 1982 suggested that follicular rupture occurs 36-38 hours after hCG administration. Although the use of hCG to induce ovulation in the treatment of infertility is common but no consensus ever exists in term of the optimal time interval between hCG injection and insemination. It was only a postulation that the follicle will rupture on the day of insemination and perhaps it will increase the chance of fertilization.

In term of number of IUI, no difference in the PR observed either using double or single IUI (Cantineau A et al, 2003). The amount of inseminated semen volume of either 0.5 ml or 3 ml had also produced a similar pregnancy rate (Do Amaral et al, 1992).

With regard to outcome of pregnancies resulting from IUI, the fecundity rate is highest during the early treatment cycles. 70% of IUI pregnancies occurred in the first two cycles of treatment. 85% of IUI pregnancies occurred during the first 4 cycles (Isaksson et al, 1997). Karlström et al in 1993 also suggested that the first treatment cycle per couple was the best predictor for evaluation of COH-IUI outcome. Therefore, this study only analyzed the outcome of first IUI cycle.

3.0 RESEARCH OBJECTIVES

3.1 GENERAL OBJECTIVE

- To compare the effectiveness of two MOH protocols in HSNZ among women undergoing COH-IUI.

3.2 SPECIFIC OBJECTIVES

- To evaluate the ovarian response using CC-rFSH and rFSH alone protocols.
- To compare the endometrial receptivity (in term of endometrial thickness) between the two protocols.
- To compare the adverse outcome of COH-IUI in both protocols
    - OHSS
    - Multiple pregnancy
    - First trimester miscarriage
    - Extrauterine pregnancy

3.3 RESEARCH HYPOTHESIS

The MOH protocol with rFSH alone is as effective as MOH protocol with combination of CC-rFSH as controlled ovarian stimulation agent in IUI cycles.

4.0 METHODOLOGY

4.1 STUDY DESIGN, LOCATION AND PERIOD OF STUDY

- This is a prospective randomised controlled trial comparing two MOH protocols with combination of CC-rFSH and rFSH alone as ovarian induction agent for IUI in infertile couples. This study was conducted in the Infertility Centre, HSNZ for a period of 1 year, from 1st October 2013 until 30th September 2014.

4.2 REFERENCE POPULATION

- All subfertile patients in Terengganu.

4.3 SOURCE POPULATION AND SAMPLING FRAME

- All subfertile women subjected to COH-IUI in Infertility Centre, HSNZ.

4.4 INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

- All patients with infertility, subjected for COH-IUI.
- 1st cycle of IUI

Exclusion Criteria

- Severe oligospermia, defined by sperm count of less than 10 million/ml by seminal fluid analysis (SFA).
- Bilateral tubal blockage

- Treatment with CC, metfomin, GnRH analogue or gonadotrophin within one month prior to randomization
- Couples who do not have complete baseline tests for infertility such as hormonal profiles, assessment of tubal patency and SFA
- Presence of evidence of ovulation prior to hCG administration

4.5 ETHICS AND CONSENT

- All couples who had fulfilled the inclusion and exclusion criteria were invited to participate in the study. Informed consent was obtained from the subjects. They were allowed to withdraw from the study at any time they wish.

  (See Appendices 1&2)

4.6 SAMPLE SIZE DETERMINATION

- As this was a pilot study, a minimum of 30 cycles of MOH protocols with CC-rFSH and 30 cycles of MOH protocol with rFSH alone were recruited in order to obtain a valid study comparison.

4.7 SAMPLING METHOD

Block Randomisation was used to determine protocol group allocation. 60 individuals were randomised into two protocol groups A (CC-rFSH protocol) and B (rFSH alone protocol), in blocks of 4. Blocks of four gave six combinations of AABB, ABAB, BBAA, BABA, ABBA and BAAB. Every combination was generated using table of random number. 15

appropriate consecutive numbers was used to assign to list of 60 participants (i.e 15 numbers x 4 blocks)

After completing randomization method describe above, a list of 60 participants was generated with assigned randomized protocol group. This list determined which protocol group every participant was allocated to. Every participant recruited was placed in consecutive manner one after the other until 60 participants were finally recruited.

4.8 STUDY METHOD

Every participant of this study was provided with IUI planner, which they brought during each visit for follicular tracking and IUI. The planner consisted of the IUI protocol and the result of follicular tracking. The IUI procedure was similar for both groups of subjects. An example of planner is as attached in Appendix 3.

4.8.1 MOH protocol

All participants recruited in this study had a transvaginal scan (TVS) on the day two of menstrual cycle as a baseline ultrasound to see any abnormal cyst and the number of antral follicles. The procedure was performed by a designated specialist blinded to the clinical data and previous ultrasound findings of the patients. A MH_2 180^o -view endovaginal transducer, which was attached to the ultrasound machine (Aloka Prosound SSD 3500, Japan), was used for this purpose.

Study subjects in the rFSH alone group started their treatment with self-administration of subcutaneous Puregon® 75 IU daily on the day five until day nine of menstrual cycle, a total of 375 IU rFSH per cycle. They were taught on the procedure prior to the scheduled time of treatment. Subjects in the CC-rFSH group started their treatment by taking CC 100mg daily for 5 consecutive days, from the second day of menses. On the day seven until day nine of menstruation, self–administered 75 IU Puregon® was administered via subcutaneous injection, giving a total of 500mg CC and 225 IU rFSH for the cycle.

4.8.2 Serial Follicular Tracking

Subjects from both groups underwent TVS monitoring by the researchers, starting on the day 10 of menstruation, then every two days to monitor the development of the follicles, until the follicles mature and reached the average size of 16 to 20mm in diameter. In this study, the sizes of mature follicles were measured and categorized into those of 16.0 - 19.9 mm and more or equal to 20 mm.

Due to inadequate facilities of our laboratory to provide quick analysis of serum oestradiol, the serial hormonal monitoring was not performed in this study. Unresponsive to ovulation induction was defined as no follicles greater than 10mm in diameter on cycle day 12. Each examination was interpreted in real time. When the follicles matured, a 10000 IU of intramuscular hCG (Pregnyl®, MSD) was administered to induce ovulation. IUI was performed 36 hours after the injection of hCG.